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Review

Complex I and energy thresholds in the brain

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ABSTRACT

Mitochondrial electron transport chain (ETC) deficiencies are thought to underlie defects in energy metabolism and have been implicated in the neurodegenerative process. In particular, reductions in complex I activities in Parkinson's disease are thought to cause bioenergetic dysfunction with subsequent degeneration of dopaminergic neurons. In terms of bioenergetics and assessing ETC-related problems in the brain, the presence of heterogeneous mitochondria has complicated matters as isolated non-synaptic mitochondria have different energy thresholds and flux control coefficients compared to isolated mitochondria of synaptic origin. The molecular mechanisms that underlie complex I deficiencies in the parkinsonian brain are unknown and are the source of intensive research. This review explores the relationship between complex I activity and energy metabolism in the brain as well as the nature of the complex I defect.

1. Complex I and Parkinson's disease

Mitochondrial dysfunction is characteristic of several neurodegenerative disorders [1] and also of the aging process [2]. Parkinson's disease is characterized by a selective decrease in dopamine in the striatum caused by a degeneration of dopaminergic neurons in the zona compacta of the substantia nigra [3,4]. Complex I (NADH:ubiquinone oxidoreductase, EC 1.6.5.3) activity is reduced by 35–40% in substantia nigra homogenates in postmortem studies of Parkinson's disease patients [5,6,7]. However, recent evidence has suggested that a complex I deficiency may be widespread in the parkinsonian brain, as the occurrence of a mutation in mitochondrial DNA in idiopathic Parkinson's disease [8], and an associated reduction in complex I activity in mitochondria from the frontal cortex [9,10] have been reported. This evidence supports the hypothesis that complex I deficiency plays a central role in the initial etiology of the disease [11,12,13].

The cause of the complex I deficiency is unknown, however, follow-up studies on Parkinson's disease patients injected with fetal tissue enriched in dopaminergic cells shows that an environment exists in the parkinsonian brain that induces the classical disease pathology in the transplanted cells [14]. It is not known if complex I activity in these cells is reduced following transplantation or if a complex I defect in surrounding cells induces the neurodegenerative condition. However, in certain *in vitro* models of the nerve terminal (synaptosomes) it has been shown that complex I deficiencies can result in large amounts of glutamate being released that may be potentially excitotoxic to surrounding cells [15]. Dopamine neurons are known to possess glutamate receptors, therefore, existing complex I deficiencies that lead to increased glutamate release may increase excitotoxic events in the parkinsonian brain.

2. Flux control analysis of oxidative phosphorylation in brain mitochondria

Analysis of complex I activities in mitochondria with the help of metabolic control analysis (MCA) has revealed some interesting findings concerning mitochondrial heterogeneity in the brain. When the relative contribution of individual mitochondrial respiratory chain complexes to the control of NAD-linked substrate (glutamate and malate) oxidative phosphorylation in mitochondria from various rat brain regions was investigated, it was found that complex I possessed a higher control over oxidative phosphorylation in synaptic mitochondria than in non-synaptic mitochondria [16–18]. As shown in Table 1, when rotenone, myxothiazol and KCN were used to titrate out complexes I, III and IV activities, respectively, while measuring oxidative phosphorylation parameters, the highest flux control coefficients were found with complex I in mitochondria of synaptic origin. It is unclear if the complex I flux control difference is due to a differing subunit compliment in both types of mitochondria at biogenesis or loss of function in mitochondria as they travel to the nerve terminal and subsequently synthesize the majority of ATP required for normal synaptic function.

Other studies have shown that respiratory chain complexes are involved in the control of mitochondrial respiration and that the distribution of the control indices of these complexes may be different, depending on the tissue from which the mitochondria were isolated [19–22]. Brain mitochondria show lowest oxidative phosphorylation efficiency with complex I substrates, measured as P/O ratios, compared to mitochondria from heart and liver [23].

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Table 1
Flux control coefficients for ETC complexes I, III and IV in brain mitochondria

	Whole brain non-synaptic mitochondria	Whole brain synaptic mitochondria	CA1 non-synaptic mitochondria	Cortex non-synaptic mitochondria	CA1 synaptic mitochondria	Cortex synaptic mitochondria
Complex I	0.14	0.29	0.13	0.11	0.30	0.32
Complex III	0.15	0.20	–	–	–	–
Complex IV	0.24	0.13	0.22	0.20	0.15	0.14

Data taken from 16, 17 and 18.

According to the summation theory for flux control [24], the sum of all the control coefficients in any pathway is equal to unity; so in the case of the three complexes described in Table 1 the total is 0.62 for synaptic mitochondria from whole brain. Other systems known to contribute to the control of mitochondrial respiration are; adenine translocator [19,25], phosphate carrier and calcium [26,27], proton leak [28], ADP-regenerating system and dicarboxylate carrier [19]. Using a top-down analysis of synaptosomal bioenergetics, it was found that the substrate oxidation system, phosphorylation system and proton leak had flux control coefficients of 0.71, 0.27 and 0.02, respectively, and that uncoupled oxygen respiration rates were significantly lower in synaptosomes from young rats when compared to synaptosomes from old rats [29].

3. Energy thresholds in brain mitochondria

While metabolic control analysis has provided insight into the control that ETC complexes have over oxidative phosphorylation, the use of specific inhibitors has also enabled researchers to model the effects of substantial ETC deficiencies on respiration rates and ATP synthesis in the mitochondrion. This concept of mitochondrial energy thresholds has been identified in many tissues [30] and has been associated with thresholds at the transcriptional and translational levels.

In preparations of brain mitochondria, titration of complex I, III, and IV activities with specific inhibitors generated threshold curves that showed the extent to which a complex activity could be inhibited before causing impairment of mitochondrial energy metabolism (Table 2). For mitochondria of non-synaptic origin, it was found that complex I, III, and IV activities need to be decreased by approximately 60, 70, and 60%, respectively, before major changes in rates of oxygen consumption and ATP synthesis were observed. Similar thresholds were observed for complexes III and IV in synaptic mitochondria suggesting that they have large reserves of activity and must be inhibited to a large extent before oxidative phosphorylation is severely compromised. These threshold effects are not restricted to brain mitochondria and have been observed in rat muscle mitochondria for complex IV [31] and III [32], where the activity must be reduced by approximately 70 and 60%, respectively, before major changes in oxidative phosphorylation occur.

However, the energy threshold for complex I is reduced to 25% in mitochondria of synaptic origin. These results suggest that, in mitochondria of synaptic origin, complex I activity has a major control of oxidative phosphorylation, such that when a threshold of 25% inhibition is exceeded, energy metabolism is severely impaired, resulting in a reduced synthesis of ATP. The significance of this finding is important in that the reported decrease of 40% in complex I activity in Parkinson's disease patients [5–7] would extrapolate to a 35–40% inhibition of ATP synthesis and respiration in the synaptic mitochondria model. There is no information on the energy thresholds for ETC complexes inside intact synaptosomes, however, they may be expected to be different considering glucose and lactate are substrates for nerve terminal bioenergetics and *in situ* mitochondria respire somewhere between state III and state IV.

Titration of complex I activity in synaptosomes, followed by depolarization with KCl or 4-aminopyridine, reduces ATP concentrations and leads to significant amounts of Ca^{2+}-independent glutamate release [15]. Extrapolation of these results to Parkinson's disease suggests that a 40% decrease in complex I activity in the substantia nigra could induce excess release of excitotoxic glutamate following action potentials. In addition, reduction of complex I activities in synaptosomes from 18 month old rats resulted in a faster release of glutamate, compared to synaptosomes from 6 month old rats, indicating that nerve terminals from aged animals as opposed to younger rats may be more susceptible to mitochondrial dysfunction [33].

Threshold effects have been described in mitochondrial diseases [30] and may be related to the balance between normal and mutant mtDNA. If the expression of this heteroplasmy of mtDNA is at the level of a given respiratory complex enzyme, then these may reinforce threshold effects observed in mitochondrial metabolism. Whether or not respiratory chain complex activities would be reduced sufficiently to seriously compromise oxidative phosphorylation would depend on the type of mitochondria affected. Due to the heterogeneous nature of brain mitochondria in which complex I thresholds in synaptic mitochondria are different to those in non-synaptic mitochondria [16,17,18], it may be possible that degeneration preferentially occurs in synapses.

4. Glutathione and complex I activity

In addition to a reduction in complex I activity in Parkinson's disease, decreased levels of glutathione have also been found in postmortem examination of the substantia nigra [34–36]. This suggests an increased oxidative stress involvement in Parkinson's disease, as glutathione is present in millimolar concentrations in mammalian cells and is considered to be a major antioxidant in the brain, capable of protecting cells from damage caused by free radicals [37].

In order to observe the consequences of glutathione depletion on mitochondrial function and to partly imitate that which is reported to occur in idiopathic Parkinson's disease, the catecholaminergic PC12 cell line was depleted of glutathione using the gamma glutamyltransferase inhibitor, L-BSO, and the effect on the complex I threshold was measured. As shown in Fig. 1, oxygen respiration exhibited a threshold effect at 30–40% inhibition of complex I activity, suggesting that PC12 cell mitochondria have a different complex I threshold to that found in synaptic and non-synaptic mitochondria. Similar thresholds for complex I activity have been reported in other cell lines, possibly reflecting the fact that mitochondria respiring *in situ*, in

Table 2
Thresholds for ETC complex control of respiration in brain mitochondria

	Whole brain non-synaptic mitochondria	Whole brain synaptic mitochondria	CA1 non-synaptic mitochondria	Cortex non-synaptic mitochondria	CA1 synaptic mitochondria	Cortex synaptic mitochondria
Complex I	72	25	60	60	25	25
Complex III	70	80	Nd	Nd	Nd	Nd
Complex IV	60	70	60	60	60	60

Data taken from 16, 17 and 18.

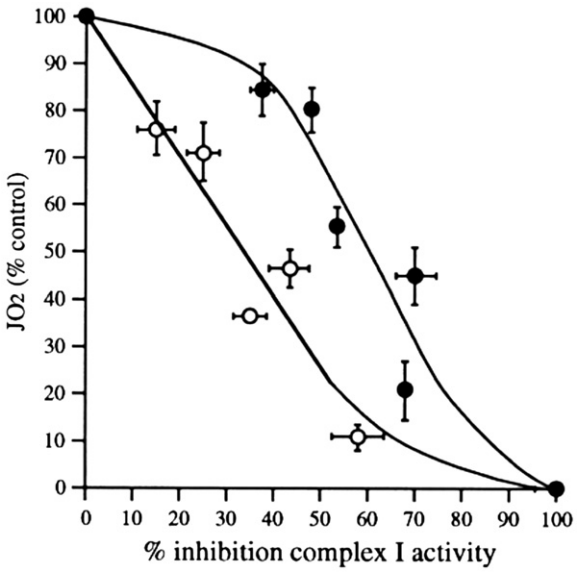

Fig. 1. Depletion of glutathione abolishes the complex I threshold in PC12 cells. PC12 cells were incubated for 18 h in the presence (○) and absence (●) of the glutathione-depleting compound, L-BSO. The percent inhibition of complex I activity was calculated and plotted against respiration rates (data taken from 18).

immortalized cell lines, have different substrates compared to isolated mitochondria. However, following the depletion of glutathione in PC12 cells the complex I threshold effect was abolished (Fig. 1).

The mechanism by which GSH causes removal of the complex I threshold in mitochondria is not known, however, glutathione is an antioxidant which protects mitochondria from lipid peroxidation [38] and when depleted may render complex I susceptible to free radical attack. Previous studies have shown that depletion of GSH induces enlargement and degeneration of brain mitochondria [39] as well as a decrease in complex IV activity in purified brain mitochondrial preparations [40]. Treatment of rat brain primary cultures with L-BSO caused decreases in complex I activity, complex II–III activity and complex IV activity of 34%, 60% and 41%, respectively [41] however when similar glutathione experiments were done with rat brain primary astrocytes, complex I activity was increased 2-fold [42] and 4-fold [43]. These results suggest that, under conditions of GSH depletion, neurons are more vulnerable to metabolic insult, which results in a compromise in mitochondrial energy metabolism.

The finding of a reduced complex I threshold in conjunction with a constitutive metabolic deficiency in dopaminergic neurons from the substantia nigra [44,45] may predispose these neurons to apoptotic or necrotic mechanisms. There is evidence that mitochondrial complex I dysfunction is a consequence of stress oxidation of critical thiol groups in the enzyme [46,47]. Similarly, reversible glutathionylation of complex I increases mitochondrial superoxide formation [48]. Thus, the implications for these results might be extendable to complex I and glutathione abnormalities that are seen in Parkinson's disease, and may underlie selective neuronal degeneration.

5. Cause of complex I deficiency — role for assembly?

As stated above, the cause of complex I deficiency in Parkinson's disease is unknown and little information is available on the nature of complex I assembly in brain mitochondria. The possibility that abnormal assembly mechanisms in the brain may exist requires further consideration and the literature on complex I structure and assembly in mitochondria from other tissues provides a starting point. Mammalian complex I is composed of 45 different subunits that assemble together at the inner mitochondrial membrane [49]. The assembly of this complex is complicated partly because of its large size and partly because its regulation by two genomes. Seven of the complex I subunits are encoded by the mitochondrial (mt) DNA and the remaining by the nuclear genes. The nuclear DNA-encoded subunits are synthesized in the cytosol and are imported into the organelle. The mtDNA-encoded subunits must assemble with the nuclear encoded subunits to generate the functional holoenzyme [49–53]. The multimeric holoenzyme is L-shaped with a hydrophobic membrane arm embedded in the mitochondrial inner membrane and perpendicular to it is a hydrophilic peripheral arm which protrudes into the mitochondrial matrix [54,55]. The 14 most conserved subunits (which form the fully functional complex I in bacteria) form the 'core' structure. The 'core' structure is also considered to be the minimal structure required for the functionality of the enzyme [56]. The function of most of the additional 31 supernumerary subunits is not yet clear but some are hypothesized to stabilize or to protect the complex from ROS damage. Several of these subunits may have an additional function. For example, the subunits NDUFA13 [also known as GRIM-19] and NDUFS1 have been implicated to have a role in apoptosis [57,58].

Assembly of complex I subunits is an intricately elaborate process which has been studied in several organisms of different evolutionary lineages. Studies in model organisms as simple as the bacteria [*Escherichia coli*] and the green algae [*Chlamydomonas reinhardtii*] to more complex eukaryotes like the fungus [*Neurospora crassa*], higher plants [*Zea mays*, *Arabidopsis thaliana*] and mammals has outlined the basic process of complex I assembly. The main consensus findings of these studies are — a] complex I assembly is not sequential but is modular which means that the subunits are not incorporated one by one into the complex but instead several discrete assembly intermediates are assembled independently and are joined in several steps to form a peripheral arm and a membrane arm; b] peripheral arm can be assembled independently of the membrane arm; c] sub-assemblies of the nuclear DNA-encoded subunits and the mtDNA-encoded subunits can be formed independently; d] in eukaryotes, a nuclear DNA-encoded scaffold of peripheral arm is attached to the inner membrane where it joins to the membrane arm intermediate [59–62].

There are many different models proposed for mammalian complex I assembly and even though there are differences between these models, a recent review draws out a general concept for complex I assembly [63]. According to a generalized model, NuoC and NuoD (bacterial homologues of NDUFS2 and NDUFS3) form the starting point of the peripheral arm assembly. Addition of NuoB, I, E, F and G (bacterial homologues of NDUFS7, NDUFS8, NDUFV1, NDUFV2 and NDUFS1) result in a large peripheral arm intermediate (~600 kDa), which is then anchored to the mitochondrial inner membrane with the help of its transporter subunits NuoH [bacterial homologue of ND1]. This membrane anchored peripheral complex extends by combining to the highly conserved hydrophobic membrane arm intermediate and results in holo-complex I (~950 kDa).

Complex I assembly is a highly dynamic process in which existing and newly synthesized subunits can be exchanged. A recent paper by Lazarou et al. [64] demonstrates that nuclear DNA-encoded subunits of complex I are incorporated into the complex much faster than the mtDNA-encoded subunits even though the nuclear DNA-encoded subunits were not rate limiting for complex I assembly. This observation leads to a suggestion that the newly synthesized nuclear DNA-encoded subunits are exchanged with the assembled ones. The authors speculate that the complex I transitions between assembled and intermediate forms may enable a mechanism that facilitates the turnover of subunits, some of which may be oxidatively damaged. Given the fact that a number of complex I subunits become oxidatively damaged in the brains of patients with Parkinson's disease [9], it is possible that an underlying basis of the disease is a decreased rate of protein import and/or assembly which slows the exchange rate of pre-existing complex I subunits for newly imported ones. This may lead to increased oxidative damage, complex I deficiency and subsequent disease.

The complex I assembly process is even more complicated by the fact that in addition to a perfect assembly, the nuclear transcription,

translation, processing, export of the subunits and then their import into the mitochondria, insertion into the membrane, stabilization and activation of the enzyme, every step requires a precise coordination. With this perspective the importance of chaperone proteins becomes evident. Chaperones are a family of proteins that assist the correct assembly of other polypeptide containing structures *in vivo*, but are not a component of the final functional structure. Examples of mutations in the assembly chaperones that lead to the inability to assemble a properly functioning complex I and thus lead to complex I deficiency diseases, have been reported [65].

In contrast to the numerous complex III and complex IV assembly chaperones known, so far only three candidate complex I assembly factors have been found. Two of these, the NDUFAF1 (the human homologue of fungal protein CIA30) and a paralogue of complex I subunit B17.2 known as B17.2-like (B17.2L) are well established and well studied complex I assembly chaperones. Ecsit (Evolutionary Conserved Signaling Intermediate in Toll pathways) is yet another recent addition to the list. The conventional role of this signaling intermediate in the Toll-pathway is in passing of a message from ligand activated plasma membrane receptor to transcription of pro-inflammatory genes. But intriguingly it has a role in mitochondrial complex I assembly [66]. This exciting finding leads to an interesting speculation that Ecsit may extend the cascade of immune response to mitochondrial level and may affect cellular energy production upon inflammation. A number of studies have linked inflammation to sporadic cases of neurodegeneration and mitochondria are known to be damaged by inflammatory processes. A very relevant example is provided by the HIV-1 induced T-cell apoptosis where viral infection downregulates the expression of complex I subunit NDUFA6 [67]. This subunit is important for complex I assembly and it associates with a 650 kDa intermediate prior to the formation of a fully assembled holoenzyme and reduction in its level would result in reduced level of functional complex I. Thus, viral infection manipulates complex I assembly process, reduces the cellular energy level to its own benefit and finally leads to the apoptosis of the T-cell. A similar link between inflammation and neurodegeneration is yet to be investigated and established.

6. Complex I and supercomplexes

Although there is a wealth of knowledge available on the structure of the individual electron transport chain complexes, their supramolecular organization into 'respirasomes' is relatively an under-studied area. This is mainly because these complexes are functionally active even when isolated as individual complexes and the detergent used to solubilize them out of the inner membrane partially destroys the supramolecular assembly. Previously, two models have been proposed for the organization of the respiratory chain. The random collision model or the 'fluid state' model, proposed by Hackenbrock [68] was accepted by many investigators in the field. According to the model complex I–IV moves freely by lateral diffusion in the inner mitochondrial membrane and electron transfer is based on random collisions of the involved components. Ubiquinone and cytochrome *c* have a diffusion rate that is faster than the bulkier complexes. This model was backed by studies on mobility of the complexes and subsequently supported by kinetic analysis of steady state respiration and saturation kinetics of electron transfer [69].

In contrast, several other lines of evidence support the 'solid state' model which promotes the idea of preferential associations and specific aggregation of the complexes: a) a mild purification and separation protocol (BN-PAGE) and immunoprecipitation results in co-purification of more than one complex [70,71]; b) flux control experiments favours the supramolecular organization of the complexes rather than their individual occurrence [72]; c) organization into supercomplexes leads to improved stabilization of individual complexes [73]; d) mutations leading to a loss of complex III prevents supercomplex formation and leads to a secondary loss of complex I [74]; e) the complexes are present *in vivo* at defined stoichiometries [75]; f) single particle electron microscopy has elucidated the 3D maps of the supercomplexes [76,77]. Inspite of the ongoing debate, the solid but dynamic model of the electron transport chain complex organization is more widely accepted now.

The supercomplexes have been identified and characterized from a broad range of organisms using BN-PAGE and sucrose gradient centrifugation. The supercomplex I–III, characterized in *Arabidopsis*, consists of complex I and dimeric complex III. The supercomplex III–IV consisting of dimeric complex III and one to two copies of monomeric complex IV, has been characterized in yeast. The largest supercomplex from bovine heart, consists of complex I plus complex III_2 plus complex IV_{1-4} and is termed the 'respirasome'. The supramolecular organization of the oxidative phosphorylation complexes into respirasomes may be of functional importance as they may enhance electron transfer rates, reduce diffusion distances of substrates by substrate channeling and increase the stability of the complexes. Although little is known about supercomplexes in the brain, the different energy thresholds and flux control coefficients described above may be related to a range of supercomplex formations in brain mitochondria.

The formation of supercomplexes I–III is facilitated by lipid component in the inner membrane. All purified preparations of mitochondrial electron transfer complexes are isolated as lipoprotein complexes. Complete extraction of phospholipids from these lipoprotein complexes reveal that cardiolipin, phosphatidylcholine and phosphatidylethanolamine are the predominant components. These phospholipids are not only responsible for dispersive solubilization and catalytic effect (specifically brought about by cardiolipin), but maybe responsible for providing a sufficiently lipophilic environment for the interaction of the oxidative phosphorylation components. The absolute requirement of cardiolipin for complex I and complex III activities suggest that this phospholipid plays a crucial role in the coupled electron transfer [78–80]. Recently using the cardiolipin lacking mutant yeast, it was demonstrated that 90% of the individual homodimers of complex III and complex IV were not organized into supercomplexes [81].

Other papers report that cardiolipin is important for the stability of supercomplexes and exposure of mitochondria to reactive oxygen species (ROS) can damage cardiolipin by the process of lipid peroxidation [82–83]. Although no direct evidence exists yet, the assumption that lipid peroxidation by ROS may disrupt the supercomplexes has pathological implications. This may be an underlying cause of aging and age-related disorders like Parkinson's disease, where damage by ROS and a reduction in mitochondrial energetic capacity due to dissociation of supercomplexes may be responsible for the deleterious consequences. A recent paper reported a mutation in human TAZ gene associated with Barth Syndrome [84]. These patients show mitochondrial morphological abnormalities and respiratory chain dysfunction. Patients with Barth syndrome lack the TAZ gene product, Tafazzin [a putative phospholipid acyltransferase], and exhibit defects in cardiolipin. Using BN-PAGE the authors demonstrated that supercomplex I/III_2 and supercomplex $I/III_2/IV$ were more labile in lymphoblasts from Barth syndrome patients. The level of complex I holoenzyme was also reduced. These interesting observations warrant investigation of ROS-induced damage to phospholipids, genetic defects in phospholipid metabolism and supercomplex instability as they may reveal new information on mechanisms that underlie neurodegenerative diseases.

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